



Attorney Docket No. 124263-1016

UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. : 10/643,160  
First Named Inventor : Dmitry M. Rudkevich  
Filing Date : August 18, 2003  
Title : METHODS, SYSTEMS AND USES FOR CALIXARENES  
Group Art Unit : 1723  
Examiner : Joseph W. Drodge  
Confirmation No. : 3568

DECLARATION UNDER 37 § CFR 1.131

I, Dmitry M. Rudkevich, declare that:

1. I am the named inventor in the above-identified U.S. Application No. 10/643,160 filed August 18, 2003.
2. I conceived and reduced to practice in the United States of America the invention as shown and described in the above-identified application prior to the date of October 25, 2002, the date of publication of Zyryanov et al. (Chem Commun 2002:2792-3).
3. Prior to October 25, 2002, I prepared a document attached as **EXHIBIT A** that represents an example of the reduction to tangible form of my mental concept of the invention as shown and claimed in the above-identified application.
4. **EXHIBIT A** is a document to Professor Atwood of the University of Missouri, Columbia, along with a copy of a manuscript entitled "Supramolecular fixation of NO<sub>2</sub> with calix[4]arenes" that describes compositions of the claimed invention and properties and methods of making the claimed invention. In particular, the second paragraph of column 1, page 2792 of Exhibit A corresponds to paragraphs [00014]-[00015] of the above-identified application and Fig. 1 on page 2792 of Exhibit A corresponds, in part, with Scheme 1 on page 15 of the originally-filed application for patent and teaches and shows a calix[4]arene compound capable of forming a complex with at least one NO<sup>+</sup> cation, wherein a detectable charge-transfer reaction occurs between the NO<sup>+</sup> cation and the calix[4]arene as set forth in the claims now pending.

5. Prior to October 25, 2002, I disclosed and reported the invention to Professor Atwood of the of the University of Missouri, Columbia, as set forth in **EXHIBIT A**.

6. Pursuant to the evidence set forth in **EXHIBIT A**, I reduced to practice the invention as described and claimed in the above-identified application in the United States of America prior to October 25, 2002.

7. It is my belief that the above-cited facts, as supported by **EXHIBIT A**, provide clear evidence of my conception and actual reduction to practice of the invention described and claimed in the above-identified application prior to October 25, 2002.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

D. Rudkevich  
Dmitry M. Rudkevich

06.07.06  
Date



EXHIBIT

A

To: Prof. Jerry L. Atwood,  
123 Chemistry Building,  
University of Missouri, Columbia,  
Missouri 65211, USA  
FAX 1 573 882 2754

Re: Supramolecular Fixation of NO<sub>2</sub> with Calix[4]arenes

Dear Dr. Atwood,

Enclosed please find the above-referenced manuscript, which I submit for publication in *Chemical Communications* as a communication. This is another paper in the series on supramolecular chemistry of gases – the project we have been working here, in Texas.

The manuscript presents our surprising findings on the interactions between simple calix[4]arenes and NO<sub>2</sub>, and unique encapsulation complexes that result. NO<sub>2</sub> is a toxic pollutant and it is also involved in a number of biochemical processes. Extensive NO<sub>2</sub> circulation in the atmosphere, industry, and agriculture requires its systematic monitoring and necessitates the development of improved methods of the NO<sub>2</sub> fixation. Our results offer a novel NO<sub>2</sub> fixation process, which employs simple calixarenes. In principle, the described complexes can be used as stable, encapsulated nitrosating and nitrating reagents. Calixarenes conveniently transmit the information about NO<sub>2</sub> binding via visible light signals. The described charge-transfer interactions are unique for NO<sub>2</sub> and would guaranty its detection in the presence of such gases as H<sub>2</sub>O, O<sub>2</sub>, HCl, SO<sub>x</sub>, NH<sub>3</sub>, and even NO. These findings open wider possibilities towards more sophisticated NO<sub>2</sub>/NO<sub>x</sub> sensing materials, including peptide-based nanostructures. The latter may be useful to detect NO<sub>x</sub> species in biological fluids, provided that the complexes with sterically hindered calixarenes are quite stable in water.

We believe that the described effects may be of interest to those working in molecular recognition, encapsulation, calixarenes in general, and also to scientists looking at novel sensing systems for ecological and biomedical monitoring.

I hope your reviewers and yourself would like our work and support the publication.

Sincerely,

Dr. Dmitry M. Rudkevich,

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## Supramolecular Fixation of $\text{NO}_2$ with Calix[4]arenes

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Reaction of  $\text{NO}_2$  with calix[4]arenes in chloroform in the presence of a Lewis acid rapidly results in intense coloration caused by the encapsulation of nitrosonium and nitronium cations.

Nitrogen dioxide ( $\text{NO}_2$ ) is a major component of so-called  $\text{NO}_x$  gases.<sup>1</sup> It is a toxic atmospheric pollutant derived from fossil fuel combustion, power plants, and large-scale industrial processes. In combination with nitric oxide (NO),  $\text{NO}_2$  is involved in various nitrosation processes in biological tissues.<sup>2</sup> Nitrosative mechanisms have been implicated in ion conductance, signal transduction, glycolysis, apoptosis, and DNA repair. Extensive  $\text{NO}_2$  circulation in the atmosphere requires not only its systematic monitoring,<sup>3</sup> but also necessitates the development of improved methods of the  $\text{NO}_2$  fixation. Here, we describe host-guest complexes, formed upon interaction between  $\text{NO}_2$  and simple calix[4]arenes. Our findings offer a novel process of  $\text{NO}_2$  utilization and may also lead towards stable, supramolecular  $\text{NO}_2$ -storing materials, and new visual sensory systems for  $\text{NO}_2$ . Calixarenes are popular building blocks for molecular containers – cavitands, carcerands, and capsules.<sup>4</sup> They provide with quite rigid,  $\pi$ -electron rich inner cavities for complexation of electron deficient guest-species. We discovered that calix[4]arenes **1**<sup>5</sup> and **2**,<sup>6</sup> possessing a cone and a 1,3-alternate conformations, respectively, reversibly interact with  $\text{NO}_2$  and trap highly reactive nitrosonium ( $\text{NO}^+$ ) cation. Bubbling  $\text{NO}_2$  through the solutions of **1** and **2** in  $\text{CHCl}_3$  resulted in instant, deep purple coloration. The UVvis spectra changed accordingly: the broad bands appeared at  $\lambda_{\text{max}} = 560$  and 512 nm, respectively. This is in a striking contrast to colorless solutions of **1** and **2**, and pale yellow solution of  $\text{NO}_2$  in  $\text{CHCl}_3$ , and implies a charge-transfer.  $\text{NO}_2$  is a popular nitrosating/nitrating agent, and its chemistry is well developed. Two molecules  $\text{NO}_2$  exist in equilibrium with  $\text{N}_2\text{O}_4$ , which may disproportionate to  $\text{NO}^+\text{NO}_3^-$  upon reacting with aromatic compounds.<sup>7</sup>

Interaction of  $\text{NO}_2$  with **1** and **2** is very dynamic, and the initial  $^1\text{H}$  NMR analysis gave rather complex, fast changing pictures. The solutions of **1,2** and excess  $\text{NO}_2$  quickly bleached within 1-2 h, yielding mixtures of *p*-nitrated calixarenes (TLC, NMR). To slow down the nitration and to identify the involved complexes, solutions of **1,2** and  $\text{NO}_2$  in  $\text{CHCl}_3$  were treated with  $\text{SnCl}_4$ . It is known that Lewis acids stabilize arene-nitrosonium charge-transfer complexes.<sup>8</sup> Subsequent precipitation with hexanes resulted in deeply colored solids, assigned to nitrosonium complexes **3** and **4** (>90% yield, Figure 1). These are extremely sensitive to moisture and temperature, and all attempts to obtain their elemental analyses and mass-spectra failed. However, **3** and **4** were stable enough to be characterized by spectroscopic techniques in dry chloroform.

Specifically, the UVvis spectra showed broad charge-transfer<sup>7</sup>

bands at  $\lambda_{\text{max}} \sim 563$  and 524 nm, and the FTIR spectra exhibited characteristic<sup>7</sup> arene- $\text{NO}^+$  stretching at  $\nu = 1923$  and 1955  $\text{cm}^{-1}$  for **3** and **4**, respectively. The  $^1\text{H}$  NMR spectra of **3** and **4** showed new sets of the calixarene signals (Figure 2). In particular, aromatic CH protons of guest-free **1** were seen as a singlet at 6.76 ppm. In nitrosonium complex **3**, these were transformed into a singlet at 7.00 ppm. The methylene bridge  $\text{CH}_2$  protons of **1** were recorded as doublets at 4.41 and 3.12 ppm ( $J = 12.5$  Hz). In complex **3**, these were seen as doublets at 4.39 and 3.43 ppm ( $J = 12$  Hz). The aromatic protons of free **2** were seen as a doublet and a triplet, 2:1, at 7.00 and 6.68 ppm, respectively ( $J = 7.5$  Hz). In nitrosonium complex **4**, these were transformed into a triplet and a doublet, 1:2, at 7.17 and 7.08 ppm, respectively ( $J = 7.5$  Hz).

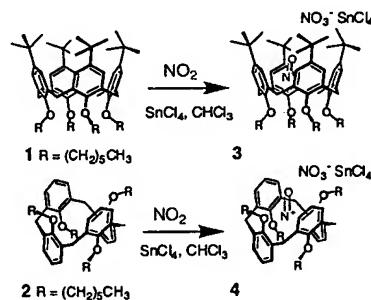


Fig. 1 Chemical fixation of  $\text{NO}_2$  with calix[4]arenes.

Independent structural evidence came from the complexation experiments between calixarenes **1,2** and commercially available  $\text{NO}^+\text{SbF}_6^-$  salt ( $\text{CDCl}_3$ , 295 K). The corresponding UVvis, FTIR and  $^1\text{H}$  NMR complexation induced changes were in agreement with the data presented above for complexes **3, 4**. According to molecular modeling, *only one*  $\text{NO}^+$  cation can fit inside the cavities **1** and **2**.

Recently, Kochi et al. described charge-transfer complexes between  $\text{NO}^+$  and structurally similar calix[4]arenes.<sup>9</sup> The cation was found encapsulated within the cavity (X-ray analysis). Owing to the fact, that two molecules  $\text{NO}_2$  may disproportionate to  $\text{NO}^+\text{NO}_3^-$ , we thus conclude that *reaction between  $\text{NO}_2$  and calix[4]arenes lead to the  $\text{NO}^+$  encapsulation*. Our spectral data are in agreement with Kochi's nitrosonium complexes.<sup>9</sup>

Moreover, in the control experiments with non-cyclic anisole (methoxybenzene), only weak, yellow coloration was observed upon exposure to  $\text{NO}_2$ . Moreover, when mesitylene-derived 1,3-alternate calixarenes,<sup>10</sup> with *blocked* cavities, were tested, no coloration was observed either; there was no indication for strong complexation in the UVvis and  $^1\text{H}$  NMR spectra. These

experiments emphasize the importance of the calixarene cavities in the described transformations and rule out the possibility of the  $\text{NO}^+$  coordination outside the cavity.

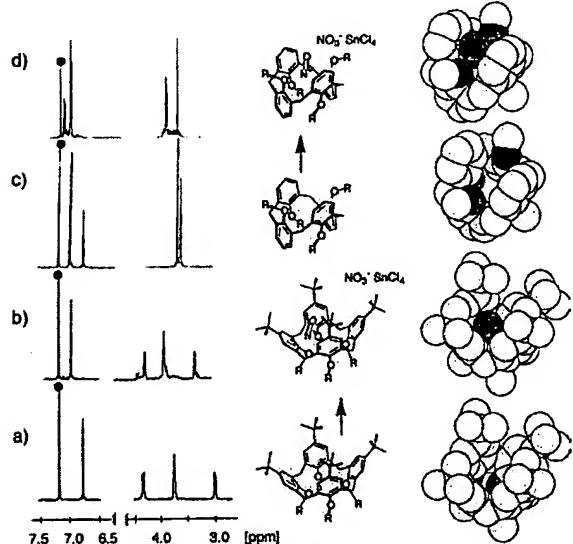


Fig. 2 Portions of the  $^1\text{H}$  NMR spectra (500 MHz,  $\text{CDCl}_3$ , 295 K) of: a) calix[4]arene 1, b) nitrosonium complex 3, c) calix[4]arene 2, d) nitrosonium complex 4. The residual  $\text{CHCl}_3$  signals are marked “\*”. Right: proposed structures 1-4; in the MacroModel 7.1 representations, long alkyl chains and hydrogens are omitted for viewing clarity.

In the experiments between calixarene 2,  $\text{SnCl}_4$  and larger (~50-fold) excess of  $\text{NO}_2$ , one more prereactive complex was detected (Figure 3), which we assigned to nitronium species 5. In the UVvis spectrum, a broad charge-transfer band at  $\lambda_{\text{max}} = 512 \text{ nm}$  was recorded. The FTIR spectrum showed stretches at  $\nu = 2356 \text{ cm}^{-1}$ , characteristic<sup>8</sup> for  $\text{NO}_2^+$  species. In the  $^1\text{H}$  NMR spectrum, complex 5 exhibits a broader doublet and a triplet, 2:1, at 7.10 and 7.00, respectively ( $J \sim 7.5 \text{ Hz}$ ). Due to their extreme reactivity, none of arene-nitronium  $\pi$ -complexes have been isolated to date,<sup>11</sup> and we attribute the stability of 5 to the encapsulation effects.

Complex 4 can be converted to 5 when larger excess of  $\text{NO}_2$  was employed. As one possible scenario, initially formed nitrosonium complex 4 yields the electron transfer complex  $[\text{2}^+ \cdot \text{NO}]^{\text{NO}_3^-}$  and releases  $\text{NO}$ . The resulting cation-radical  $2^+ \text{NO}_3^-$  reacts with an excess of free radical  $\text{NO}_2$ , producing  $[\text{2}^+ \text{NO}_2]^{\text{NO}_3^-}$  and further charge-transfer nitronium complex 5 ( $[\text{2}^+ \text{NO}_2]^{\text{NO}_3^-}$ ). Judging from the intense coloration, the calixarene walls in 5, most probably, encapsulate linear  $\text{NO}_2^+$  cation, however more experiments are needed to further prove this. At this stage, the structure of 5 was independently confirmed by complexation between 2 and  $\text{NO}_2^+\text{SbF}_6^-$  salt in  $\text{CDCl}_3$ . The corresponding UVvis, FTIR and  $^1\text{H}$  NMR spectra were similar to those of complex 5.

We then reexamined the initial reaction between 1,2 and  $\text{NO}_2$  by UVvis and  $^1\text{H}$  NMR techniques. As an excess  $\text{NO}_2$  was passed through the solution of 1, spectral features of nitrosonium complex  $[\text{1}^+ \text{NO}]^{\text{NO}_3^-}$  were clearly recorded. For 2, no signals for nitrosonium complex  $[\text{2}^+ \text{NO}]^{\text{NO}_3^-}$  were seen, but nitronium complex  $[\text{2}^+ \text{NO}_2]^{\text{NO}_3^-}$  was detected. Apparently, while formed the nitrosonium species quickly react with an excess  $\text{NO}_2$ . The colored solutions quickly bleached, yielding *p*-nitrated calixarenes.

Addition of  $\text{H}_2\text{O}$  or alcohols to the  $\text{CHCl}_3$  solutions of 3-5 resulted in the complete dissociation and recovery of calixarenes 1,2 (PTLC, UVvis,  $^1\text{H}$  NMR).

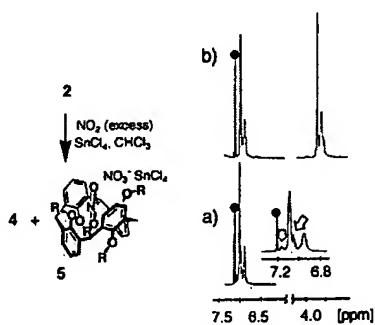


Fig. 3 Nitronium complex 5. Right: portions of the  $^1\text{H}$  NMR spectra (500 MHz,  $\text{CDCl}_3$ , 295 K) of: a) a mixture of nitrosonium 4 and nitronium 5 complexes, ~1:6. The signals for 4 are marked by arrows. b) complex 5.

Noteworthy,  $\text{H}_2\text{O}$  decolorates 4 instantly, but takes several minutes to decompose complex 3. Apparently, *t*-Bu groups at the upper rim of the latter pose significant steric hindrance and protect the encapsulated  $\text{NO}^+$  species. Such stability of the arene- $\text{NO}^+$  complex is without precedent. Primary and secondary amides also bleached the solutions of 3 and 4.<sup>4</sup>

In summary, a novel  $\text{NO}_2$  fixation process is now available, which employs simple calixarenes. In principle, the resulting complexes can be used as stable, encapsulated nitrosating and nitrating reagents. Calixarenes conveniently transmit the information about  $\text{NO}_2$  binding via visible light signals. The described charge-transfer interactions are unique for  $\text{NO}_2$  and would guarantee its detection in the presence of such gases as  $\text{H}_2\text{O}$ ,  $\text{O}_2$ ,  $\text{HCl}$ ,  $\text{SO}_x$ ,  $\text{NH}_3$ , and even  $\text{NO}$ . These findings open wider possibilities towards more sophisticated  $\text{NO}_2/\text{NO}_x$  sensing materials, including peptide-based nanostructures. The latter may be useful to detect  $\text{NO}_x$  species in biological fluids, provided that the complexes with sterically hindered calixarenes are quite stable in water. We are currently exploring all these directions.

Financial support is acknowledged from the Donors of The Petroleum Research Fund, administered by the American Chemical Society, and the University of Texas at Arlington.

## Notes and references

- † Even slight excess of  $\text{NO}^+\text{SbF}_6^-$  results in the complete complex formation in  $\text{CDCl}_3$ , and no free calixarenes 1,2 were observed after equilibration;  $K_{1,2} > 10^6 \text{ M}^1$  for both complexes was estimated. The experimental details will be given in a full paper.
- ‡ Mixing nitronium complex 5 and  $\text{AlkC(O)}\text{NH}_2$  ( $\text{Alk} = \text{Me, } t\text{-Bu}$ ) in  $\text{MeCN}$  at 295 K resulted in the nitro derivatives  $\text{RC(O)}\text{NHNO}_2$ .
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